**Supporting Information**

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# Computational Study and Biological Evaluation of Isolated Saponins from the Fruits of *Gleditsia aquatica* and *Gleditsia caspica*

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| Table of Contents | Page |
|---|---|


 

 

## 1. Materials and Methods

### *1.1. General Experimental Procedures*

Optical rotations were determined using a Perkin-Elmer Model 341 LC polarimeter (Perkin-Elmer, Waltham, MA, USA); UV spectra were determined with a Hitachi 340 spectrophotometer; IR spectra were carried out on a Shimadzu Infrared-400 spectrophotometer (Shimadzu, Kyoto, Japan) or on a Nicolet 205 FT-IR spectrometer connected to a Hewlett–Packard ColorPro. Plotter; $^{1}H$ and $^{13}C$-NMR measurements were obtained with a Bruker Avance III spectrometer operating at 500 MHz (for $^{1}H$) and 125 MHz (for $^{13}C$) or with a Bruker Avance DRX-850 MHz (for $^{1}H$) (Bruker BioSpin, Billerica, MA, USA) or a Bruker ARX-500, and a Jeol JNM ECA 500 NMR spectrometers operating at 500 MHz (for $^{1}H$) and 125 MHz (for $^{13}C$) in DMSO-$d_6$ or $CD_3OD$ or pyridine-$d_5$ solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz; $^{13}C$ multiplicities were determined by the DEPT pulse sequence (135°). DQF-COSY, HSQC, HMQC, TOCSY, $^{1}H$-$^{13}C$-HSQC-TOCSY, HMBC, ROESY and NOESY NMR experiments were carried out using a Bruker AV-500 spectrometer a Bruker ARX-500 high field spectrometers; HRESIMS (positive ion acquisition mode) was carried out on a Bruker Impact II (Bruker Daltonics, USA) or on a Bruker Bioapex-FTMS with electrospray ionization mass spectrometers; ESIMS was carried out on a TSQ700 triple quadruple instrument (Finnegan, Santos, CA, USA) mass spectrometer; EIMS was carried out on Scan EIMS-TIC, VG-ZAB-HF, X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.); Si gel (Si gel 60, Merck) and Polyamide (ICN Biomedicals) were used for open column chromatography; Flash column liquid chromatography was performed using J.T. Baker glassware with 40 μm Si gel (Baker) and Sepralyte C18 (40 μm); Semi-prep HPLC was performed on Waters Semi-prep HPLC (Waters Corp, Singapore), ODS column (Bondapak, 10 μm, 125 A, 7.8 mm x 300 mm Prep column), Detectors: PDA at 220 nm and refractive index, Flow rate: 2.0 mL/min, Sample volume (loop): 100 mL; TLC was carried out on precoated silica gel 60 F254 (Merck) plates; Developed chromatograms were visualized by spraying with 1% vanillin-$H_2SO_4$, followed by heating at 100 for 5 min.

### *1.2. Extraction and Isolation*

#### *1.2.1. Extraction and Isolation of Aquaticasaponin A and B*

The fruits of *G. aquatica* were dried and grind (3.0 kg) then exhaustively extracted with 95% ethanol. The ethanol is evaporated and the dried ethanolic extract (230.0 g) was dissolved in water and successively partitioned with petroleum ether (16.5 g), EtOAc (7.0 g) and *n*-BuOH (47.0 g). The *n*-BuOH fraction was further fractionated on a polyamide column [100% $H_2O$, 25, 50, 75, and 100 %MeOH]. The $H_2O$ fraction (28.0 g) was chromatographed on Si gel and Si gel flash CC ($CHCl_3$–MeOH: 90:10-70:30) to give four sub-fractions of A-D. Sub-fraction D (4.3 g) was chromatographed over Sepralyte $RP_{18}$ CC (MeOH–$H_2O$: 50:50-60:40) to give D1 (1.9 g), and D2 (2.0 g). Fraction D1 and fraction D2 were separately rechromatographed over Si gel and Si gel flash CC ($CHCl_3$–MeOH: 80:20-75:25). Fraction D1 give four fractions of D1a- D1d and fraction D2 give three fractions of D2a-D2c. Fractions D1c (210 mg) and D2b (425 mg) were separately subjected to Sepralyte $RP_{18}$ CC (MeOH–$H_2O$: 57:43-59:41) and finally purified by Sephadex LH-20 CC (MeOH) to afford compound **1** (50 mg) and compound **2** (40 mg), respectively.

#### *1.2.2. Extraction and Isolation of Caspicaoside L and M*

The fruits of *G. caspica* were dried and grind (2.0 Kg) then exhaustively extracted with 95% ethanol. The ethanol is evaporated and the dried ethanolic extract (390.0 g) was dissolved in water and successively partitioned with petroleum ether (14.0 g), EtOAc (11.0 g) and *n*-BuOH (180.0 g). After dissolving the *n*-BuOH fraction in a minimum amount of methanol, a large excess of acetone was added to precipitates a crude saponin mixture. By repeatedly chromatographing the crude saponin

 

mixture on Si gel and Si gel flash columns ($CHCl_3$–MeOH: 90:10-60:40) give four fractions of A-D. Fraction A (1.0 g) was chromatographed on Sepralyte $C_{18}$ flash column (30-90% MeOH in $H_2O$) to give four sub-fractions of A1-A4. Sub-fraction A3 (250 mg) was applied to a column of Si gel eluted with $CHCl_3$–MeOH: 95:5-85:15, followed by semi-preparative HPLC purification using 80% MeOH in $H_2O$ as an eluent to give compound **4** (14 mg). Similarly, fraction C (4.0 g) was chromatographed on Sepralyte $C_{18}$ flash column (30-70% MeOH in $H_2O$) to give three sub-fractions of C1-C3. By the same method, sub-fraction C2 (900 mg) was chromatographed on Sepralyte $C_{18}$ flash column (60% MeOH in $H_2O$) to give two fractions of C2a and C2b. Fraction C2b (250 mg) was repeatedly applied to a column of Si gel eluted with $CHCl_3$–MeOH-80:20, followed by semi-preparative HPLC purification using 70% MeOH in $H_2O$ as an eluent to afford compound **3** (8 mg).

*1.2.3. Antimicrobial Assay*

Compounds **1**–**4** were tested for antimicrobial activity against *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 33591 (MRSA), *Mycobacterium intracellulare* ATCC 23068, and *Candida glabrata* ATCC 90030. Ciprofloxacin and amphotericin B were used as positive controls for bacteria and fungi, respectively.

*1.2.4. Cytotoxicity assay*

*In vitro* cytotoxic activity was determined against four human cancer cell lines; skin melanoma (SK-MEL), epidermal carcinoma (KB), breast carcinoma (BT-549), ovarian carcinoma (SKOV-3) and two non-cancerous kidney cell lines (LLC-PK11 and Vero). All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

*1.2.5. Molecular Docking*

The crystal structure of human tetraspanin CD81 in complex with cholesterol was downloaded from the protein data bank (PDB: 5TCX). Protein Preparation Wizard was used to process the protein structure and correct any structural error before the docking step. Bond orders, missing hydrogen atoms, missing amino acids and side chains were corrected. The hydrogen bond network was optimized at physiologic pH and the protein was relaxed through a genera restrained minimization. The docking grid was prepared by selecting the ligand coordinates as the centroid of the docking region. Van der Waals radius scaling was used to soften the potential of non-polar parts of the receptor to add some flexibility during the docking step. A scaling factor of 0.85 was employed. The receptor hydroxyl and thiol groups could rotate in order to optimize the hydrogen bonds with docked ligands. Because the isolated compounds contain glycosidic linkages which are liable for enzymatic hydrolysis, we used only the aglycon components to check their fitting. OPLS3e force field was used for ligand preparation. Ionization, protonation and tautomerization states were generated at physiologic pH and the stereochemical information was determined from the 3D structure. Only one low energy conformer was generated for each lignad at the end of the preparation step. Ligand docking was performed by using Glide standard precision

 

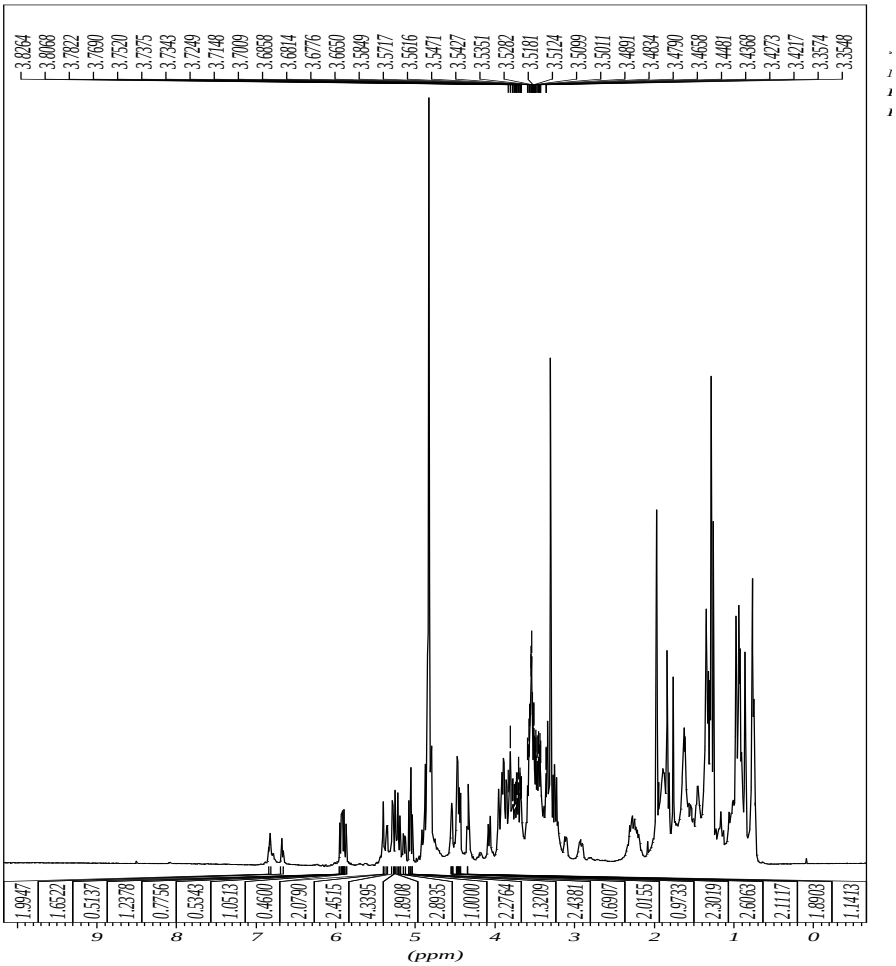


**Figure S1**: $^{1}$H NMR spectrum of compound **1** (*$CD_3OD$*, 500 MHz)

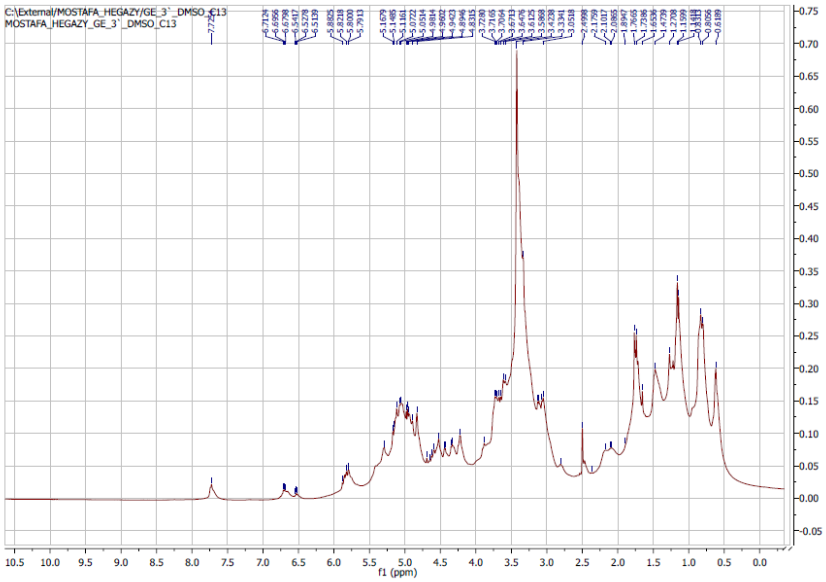


**Figure S2**: $^{1}$H NMR spectrum of compound **1** (DMSO-*$d_6$*, 500 MHz)

 

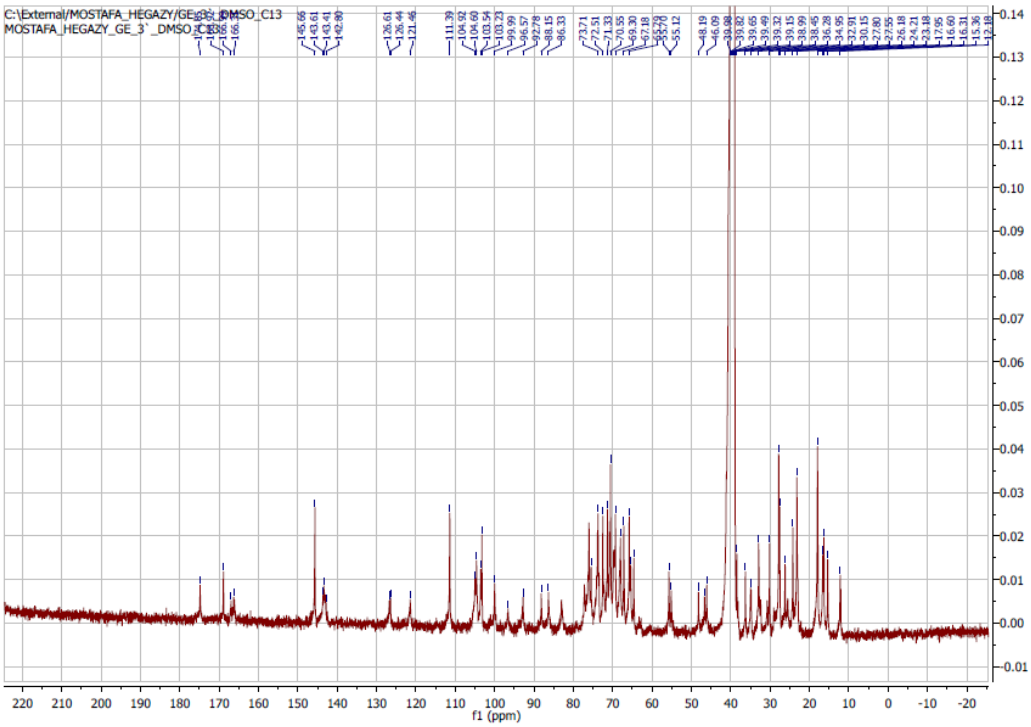


**Figure S3**: $^{13}$C NMR spectrum of compound **1** (DMSO-$d_6$, 500 MHz)

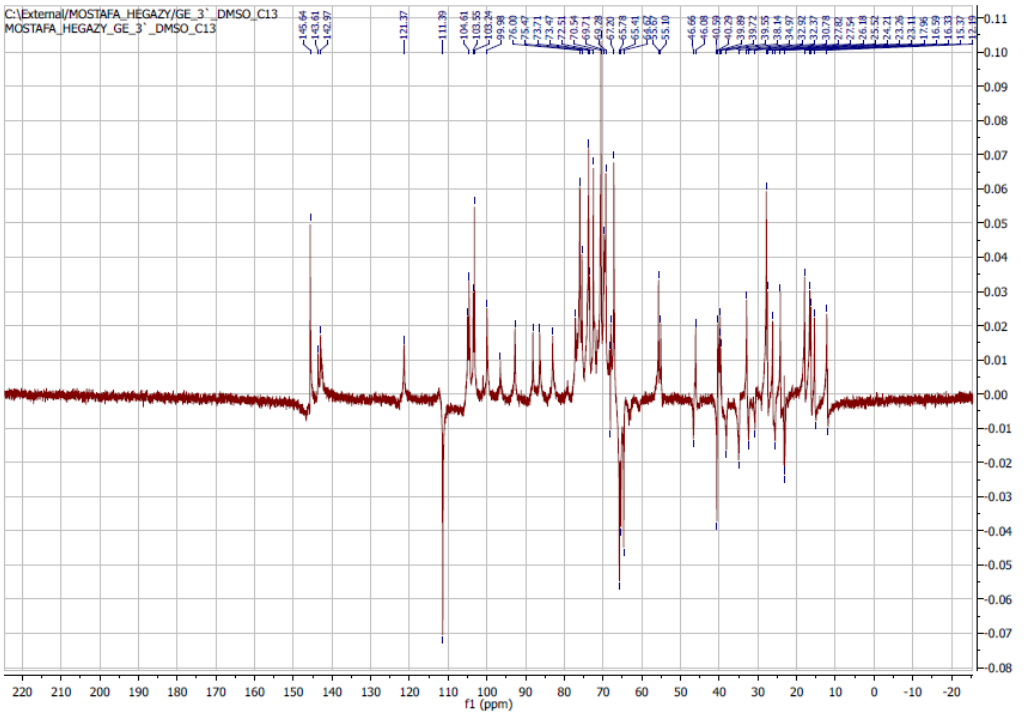


**Figure S4**: $^{13}$C-DEPT NMR spectrum of compound **1** (DMSO-$d_6$, 500 MHz)

 

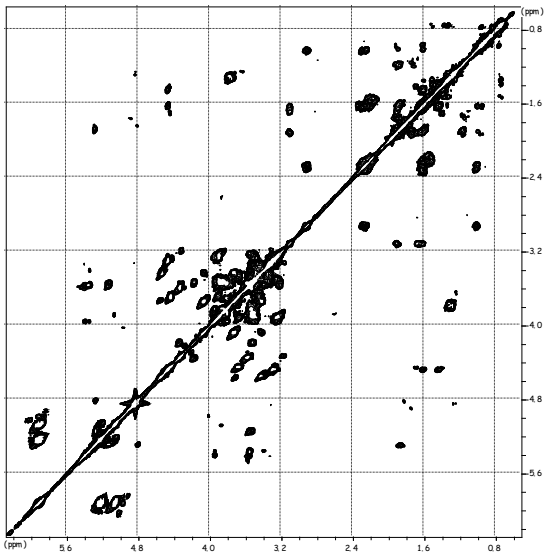


**Figure S5**: COSY spectrum of compound **1**

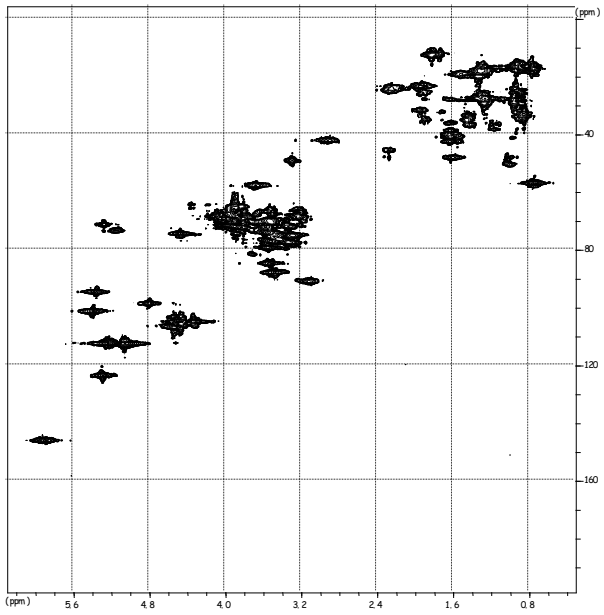


**Figure S6**: HMQC spectrum of compound **1**

 

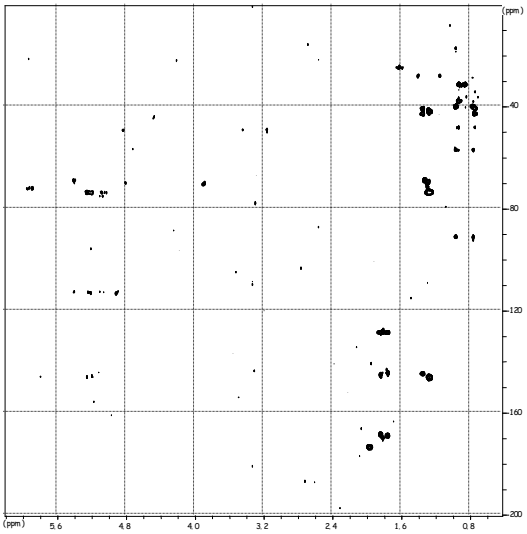

**Figure S7**: HMBC spectrum of compound **1**

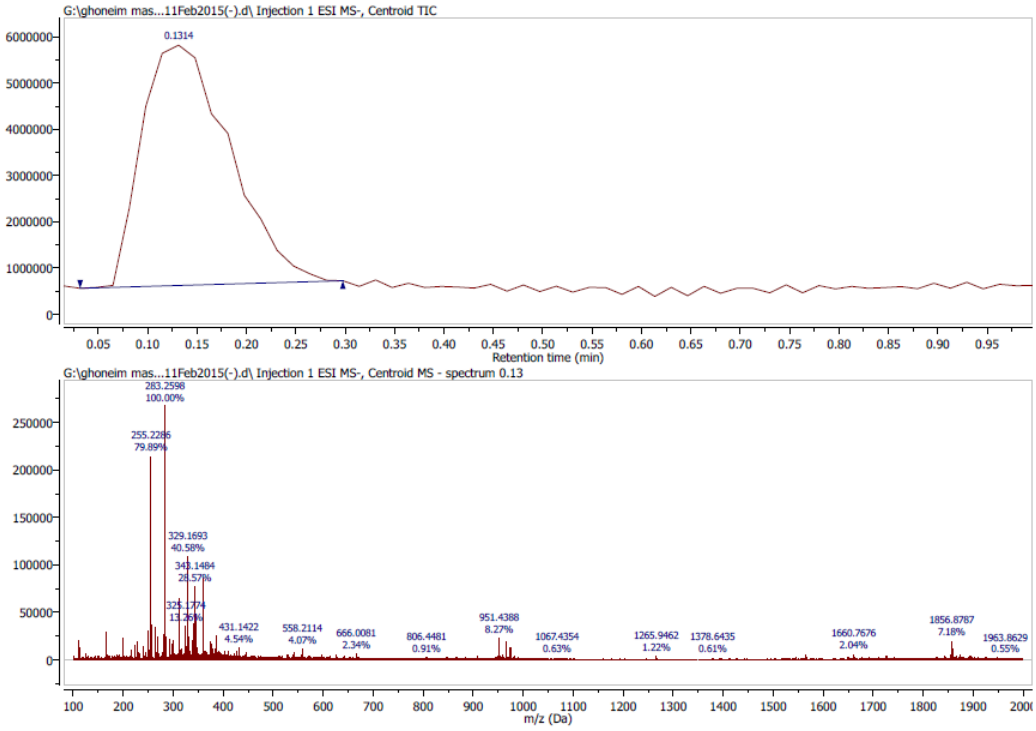


**Figure S8**: HRESI spectrum of compound **1**

 

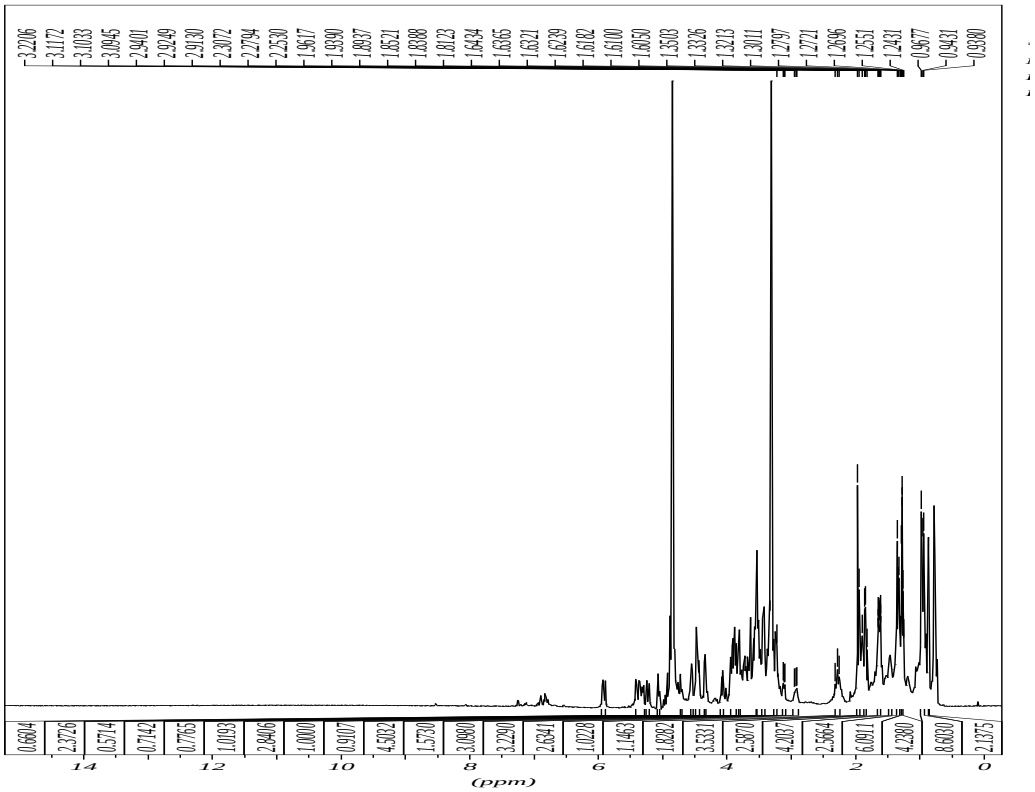


**Figure S9**: $^{1}$H NMR spectrum of compound **2** (*$CD_3OD$*, 500 MHz)

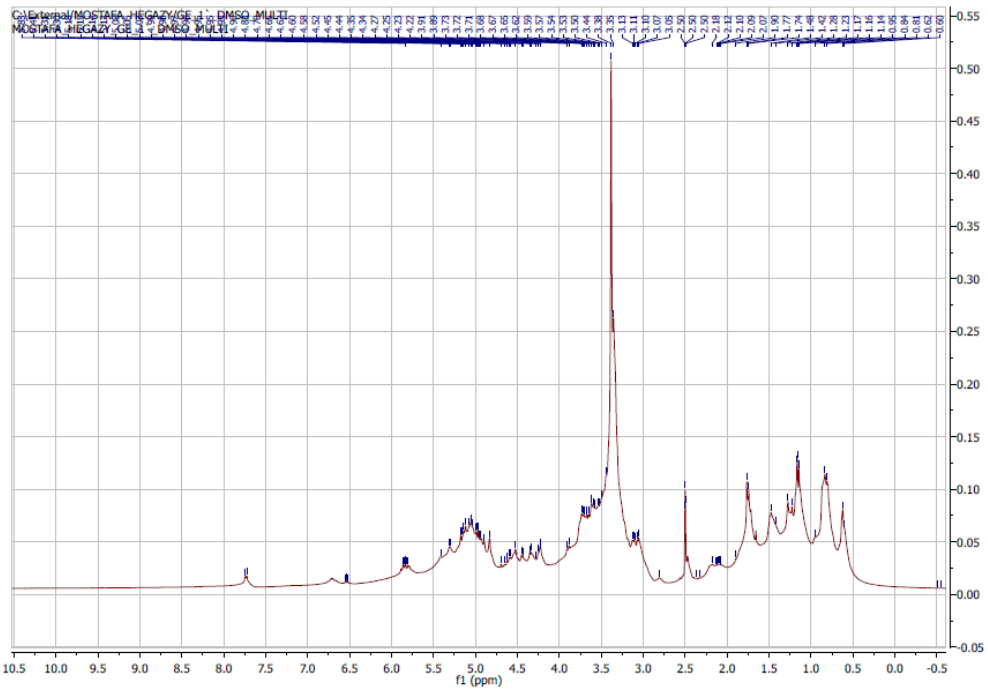


**Figure S10**: $^{1}$H NMR spectrum of compound **2** (DMSO-*$d_6$*, 500 MHz)

 

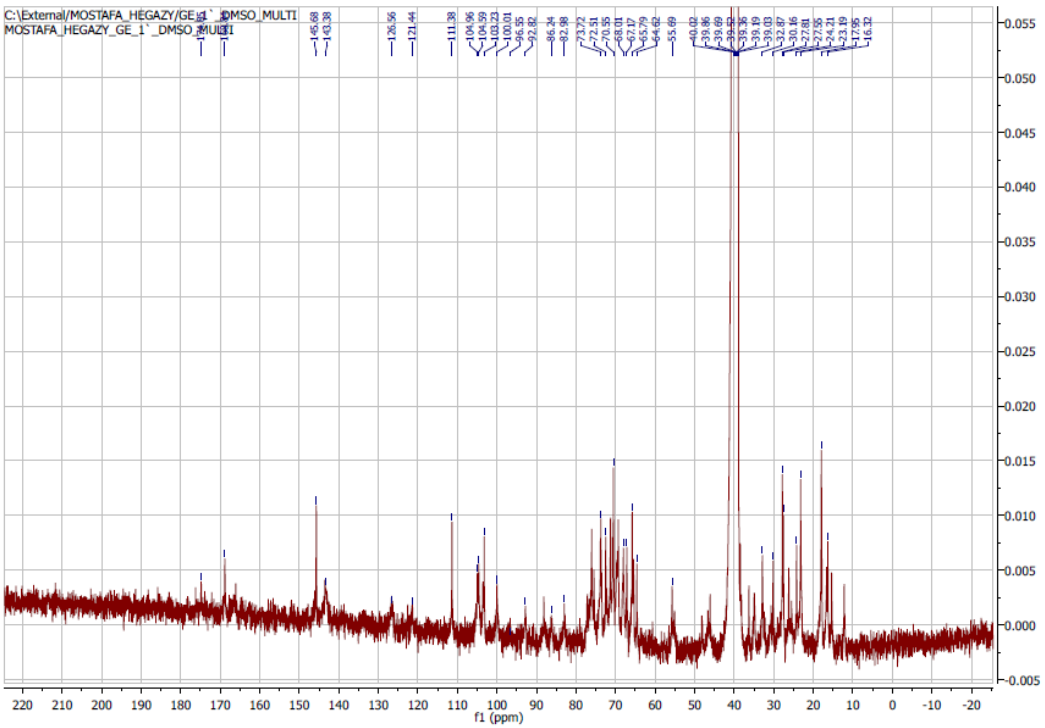


**Figure S11**: $^{13}$C NMR spectrum of compound **2** (DMSO-$d_6$, 500 MHz)

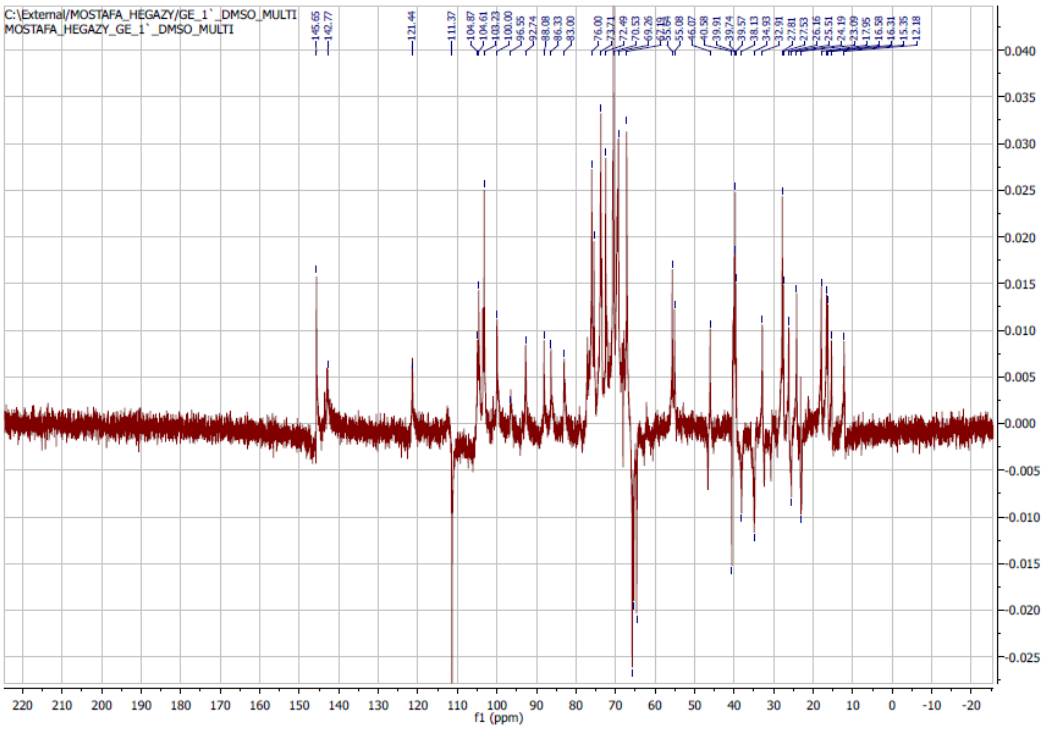


**Figure S12**: $^{13}$C-DEPT NMR NMR spectrum of compound **2** (DMSO-$d_6$, 500 MHz)

 

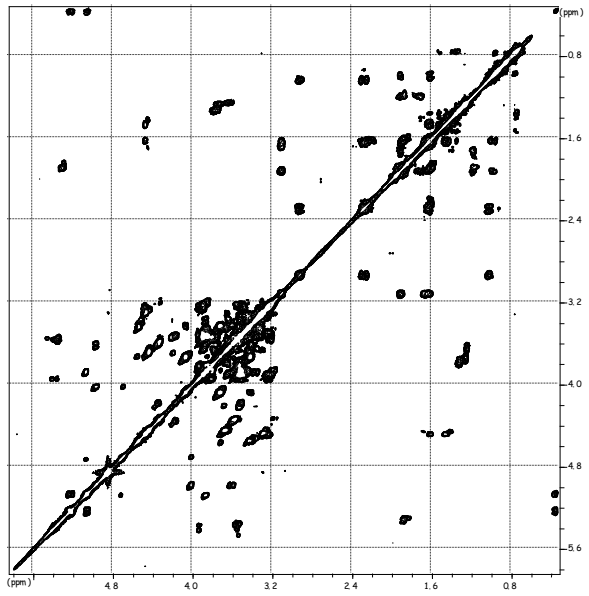


**Figure S13**: COSY spectrum of compound **2**

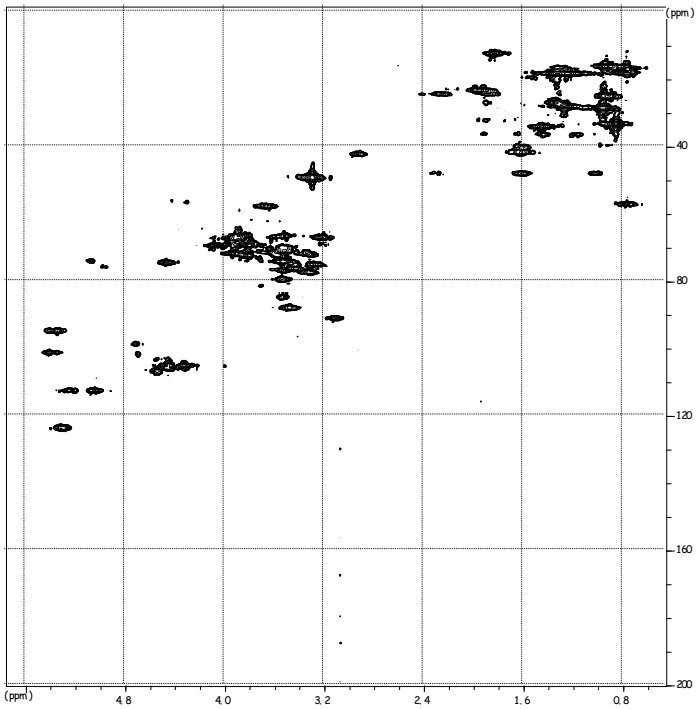


**Figure S14**: HMQC spectrum of compound **2**

 

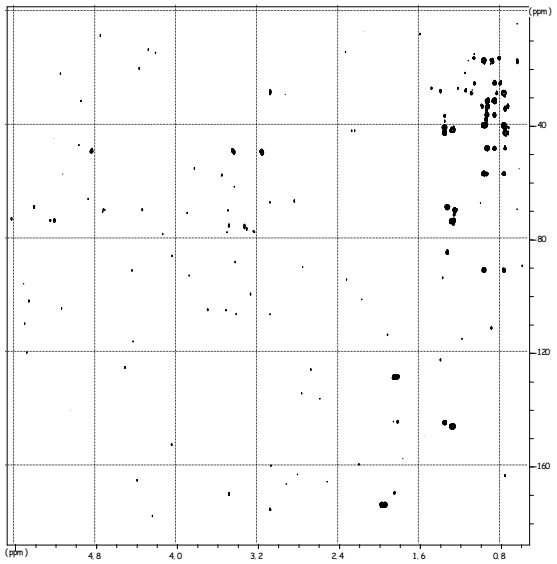


**Figure S15**: HMBC spectrum of compound **2**

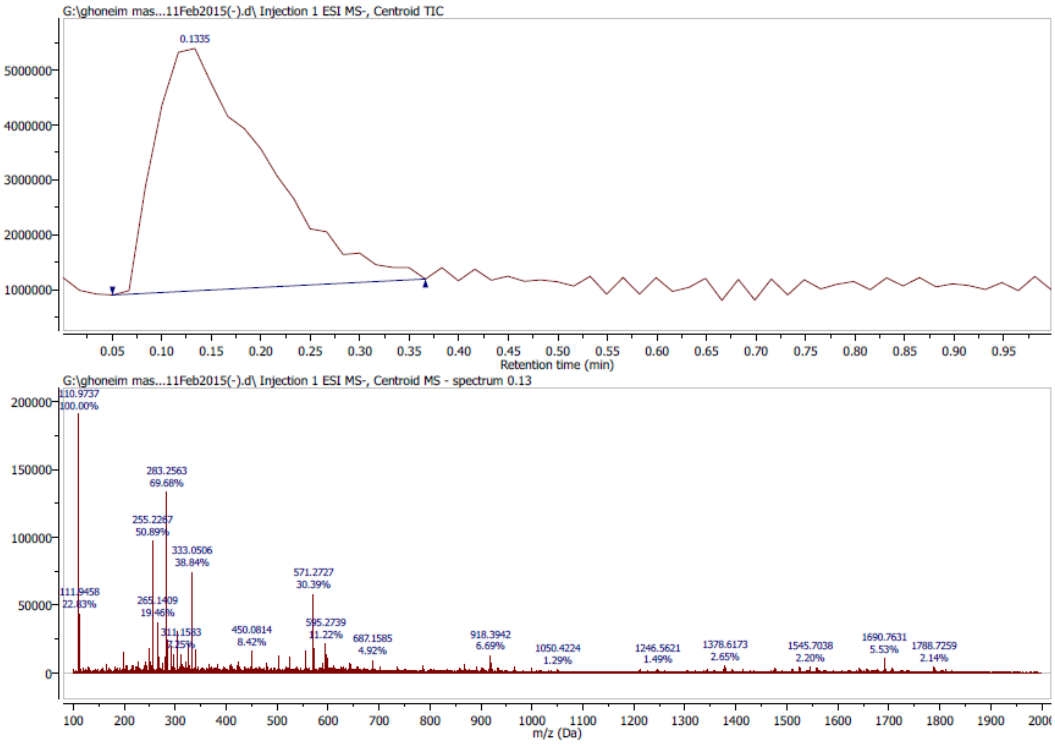


**Figure S16**: HRESI spectrum of compound **2**

 

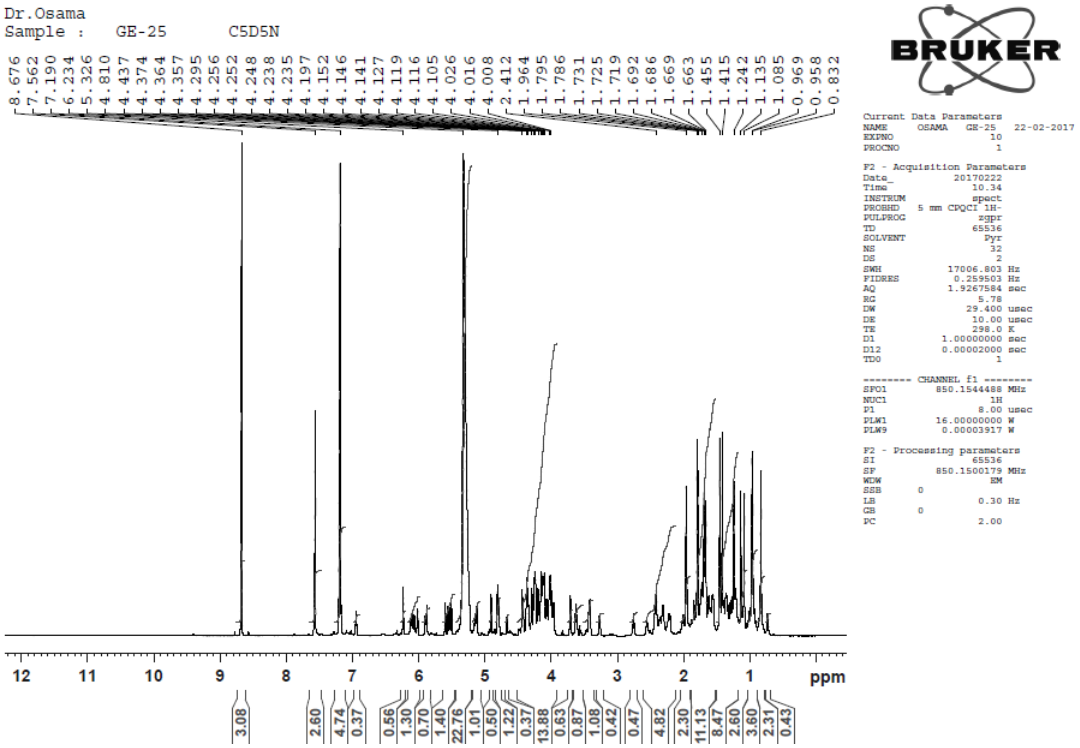


**Figure S17:** $^{1}$H NMR spectrum of compound **3** (Pyridine-$d_5$, 850 MHz)

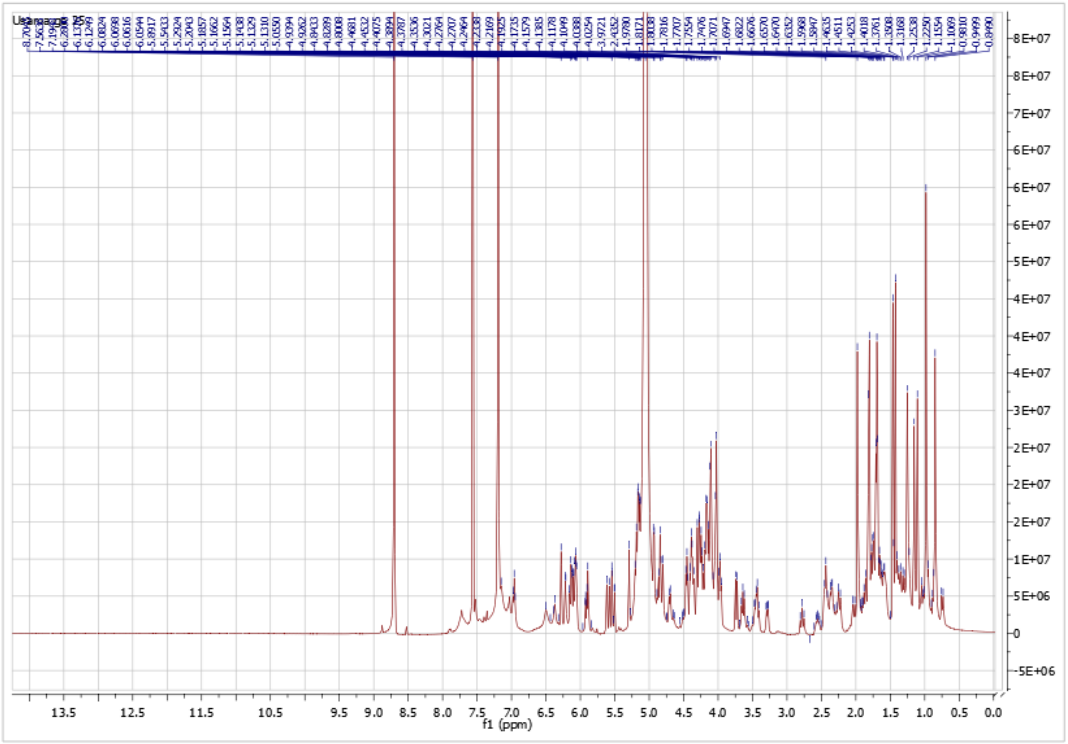


**Figure S18:** $^{1}$H NMR spectrum of compound **3** (Pyridine-$d_5$, 500 MHz)

 

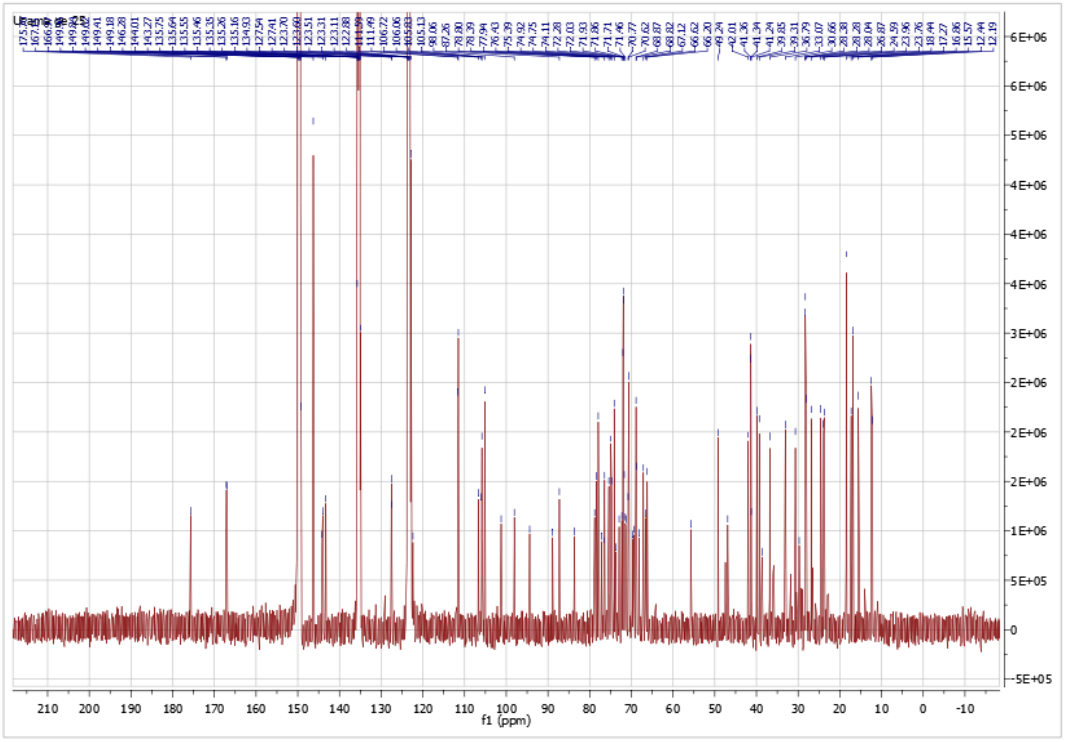


**Figure S19:** $^{13}$C NMR spectrum of compound **3** (Pyridine-$d_5$, 500 MHz)

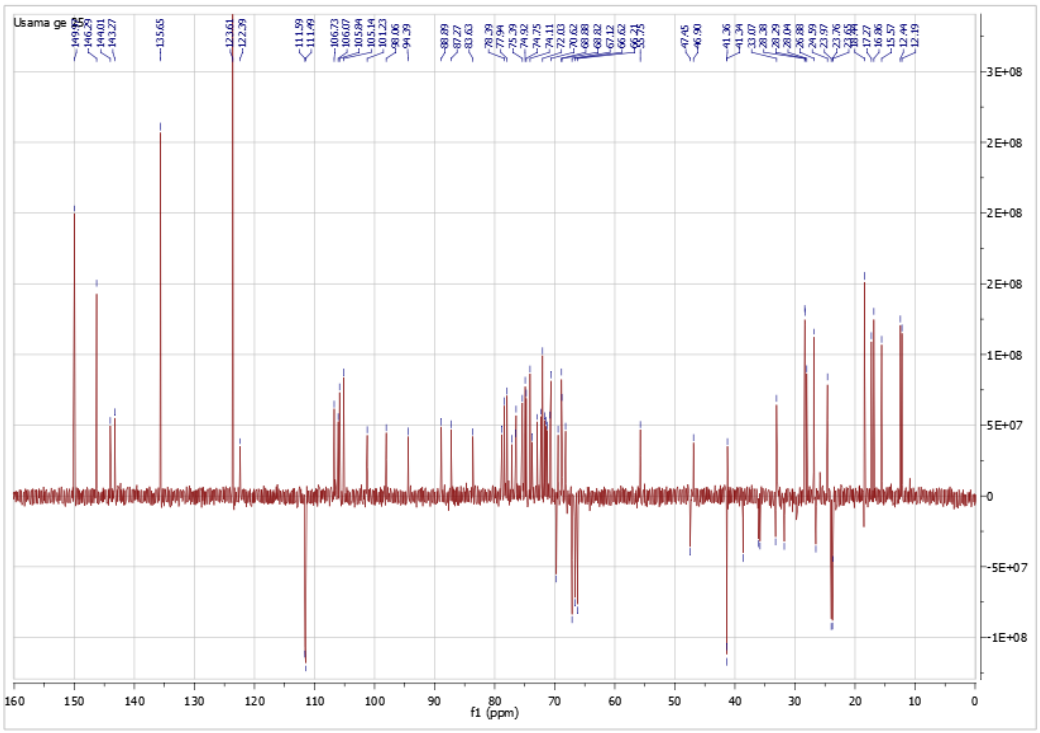


**Figure S20:** $^{13}$C-DEPT NMR spectrum of compound **3** (Pyridine-$d_5$, 500 MHz)

 

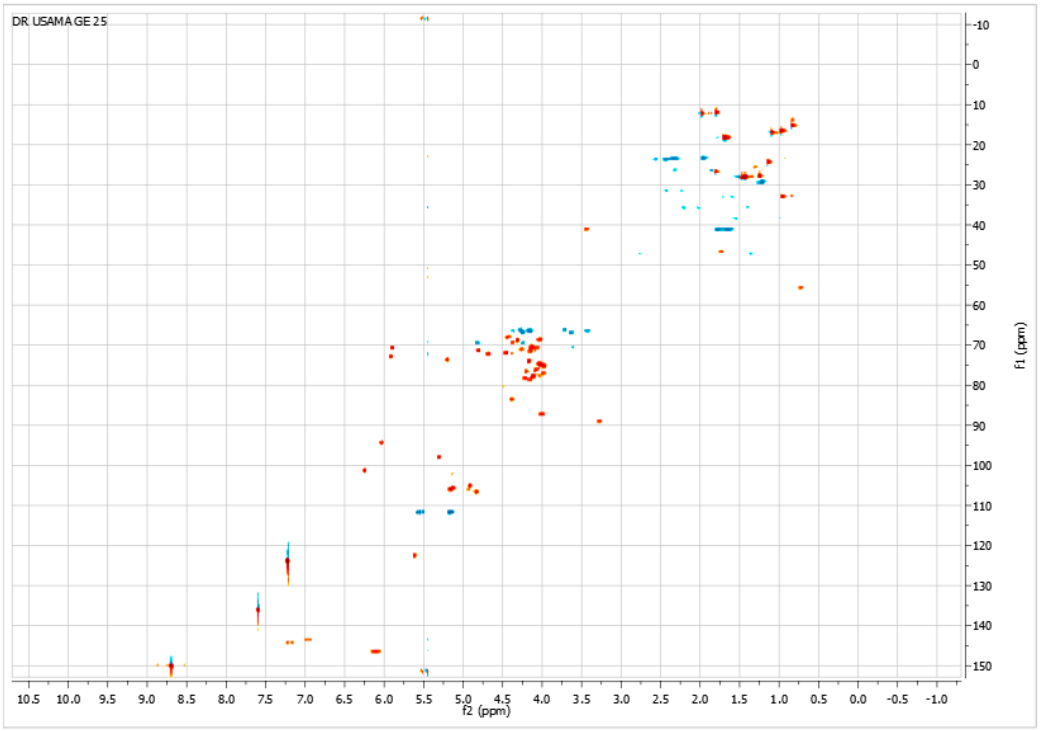


**Figure S21:** HSQC spectrum of compound **3**

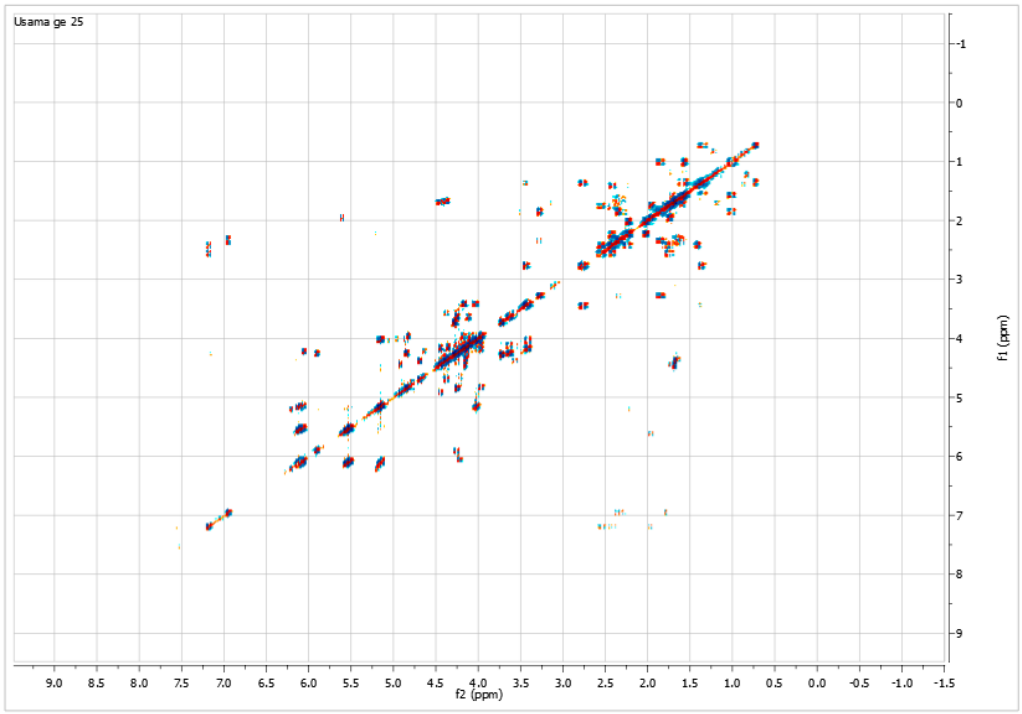


**Figure S22:** COSY spectrum of compound **3**

 

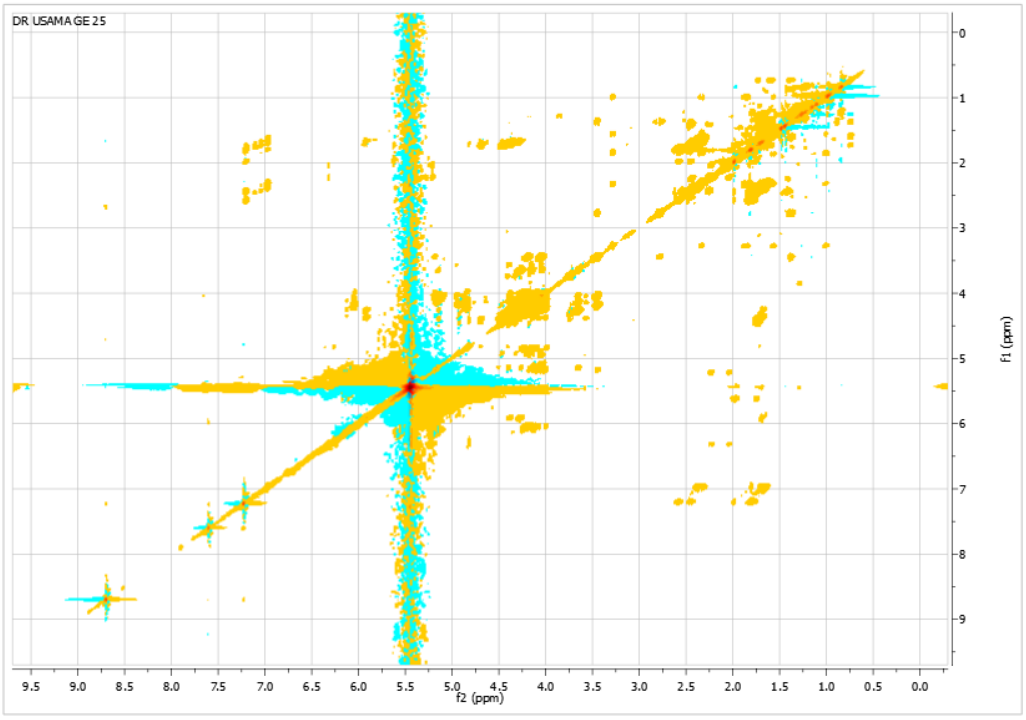


**Figure S23:** TOCSY spectrum of compound **3**

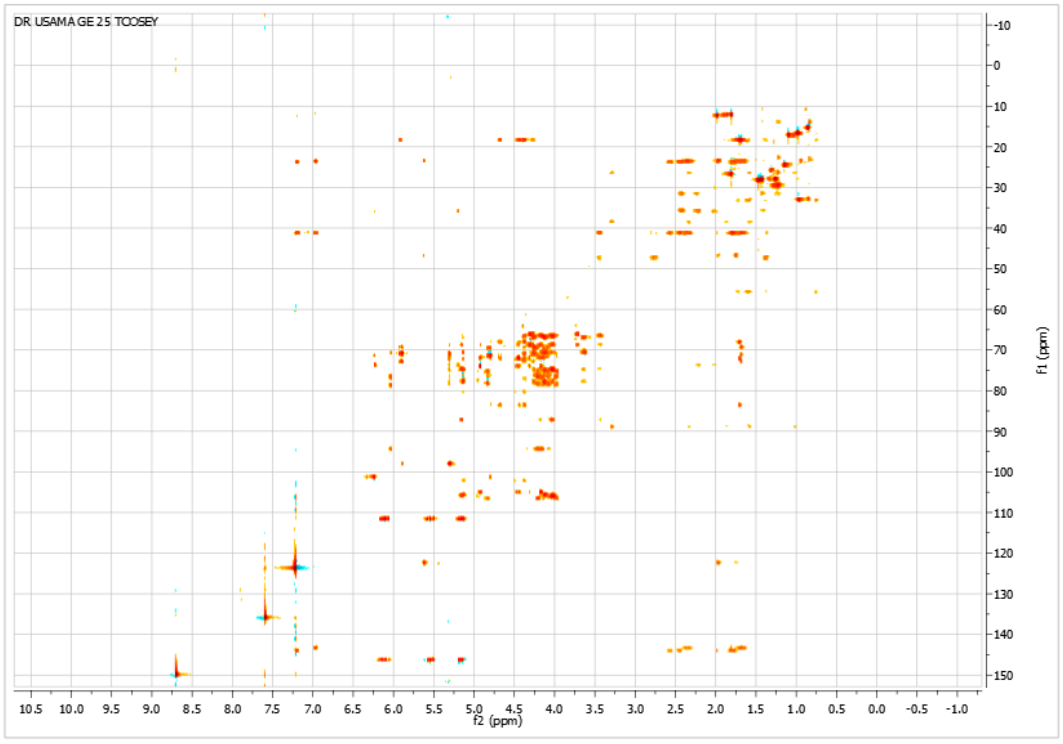


**Figure S24:** HSQC-TOCSY spectrum of compound **3**

 

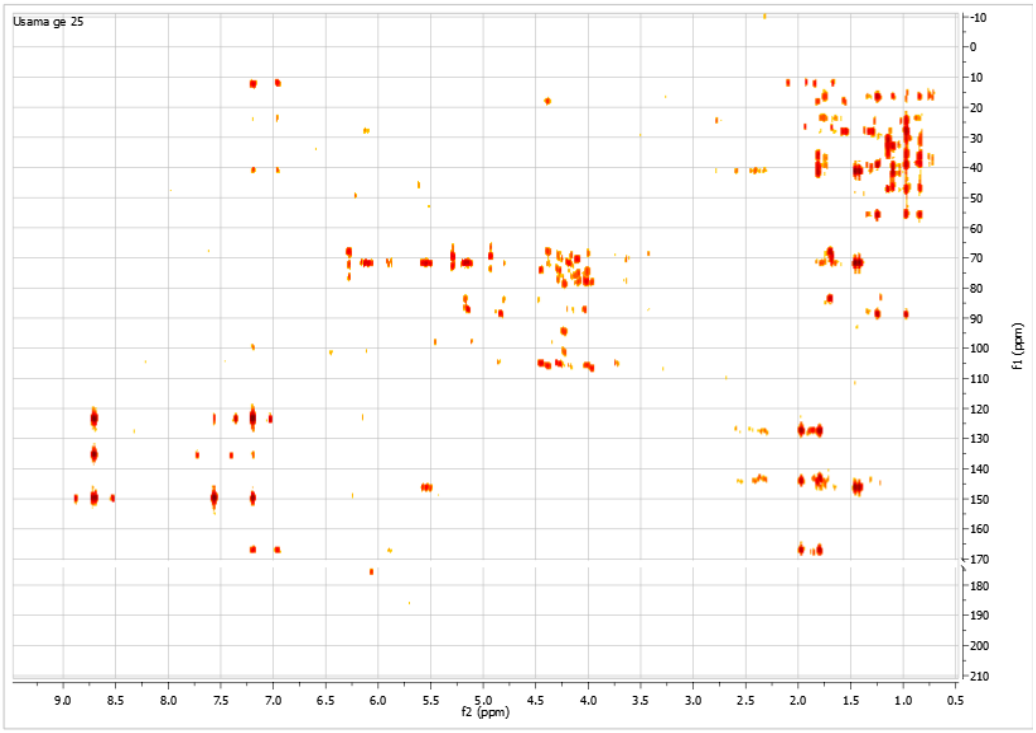


**Figure S25:** HMBC spectrum of compound **3**

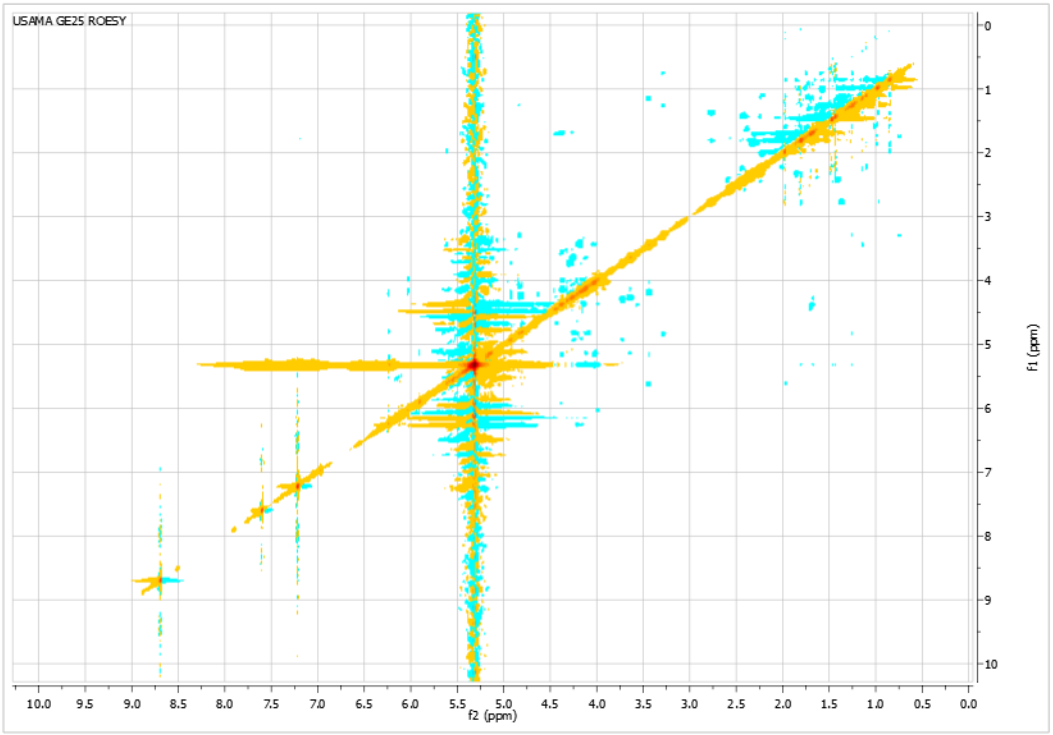


**Figure S26:** ROESY spectrum of compound **3**

 

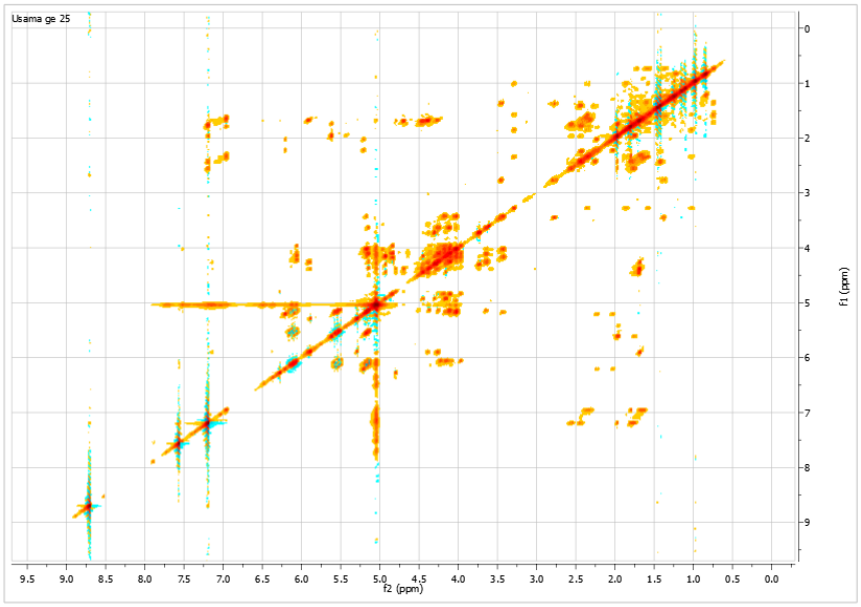


**Figure S27:** NOESY spectrum of compound **3**

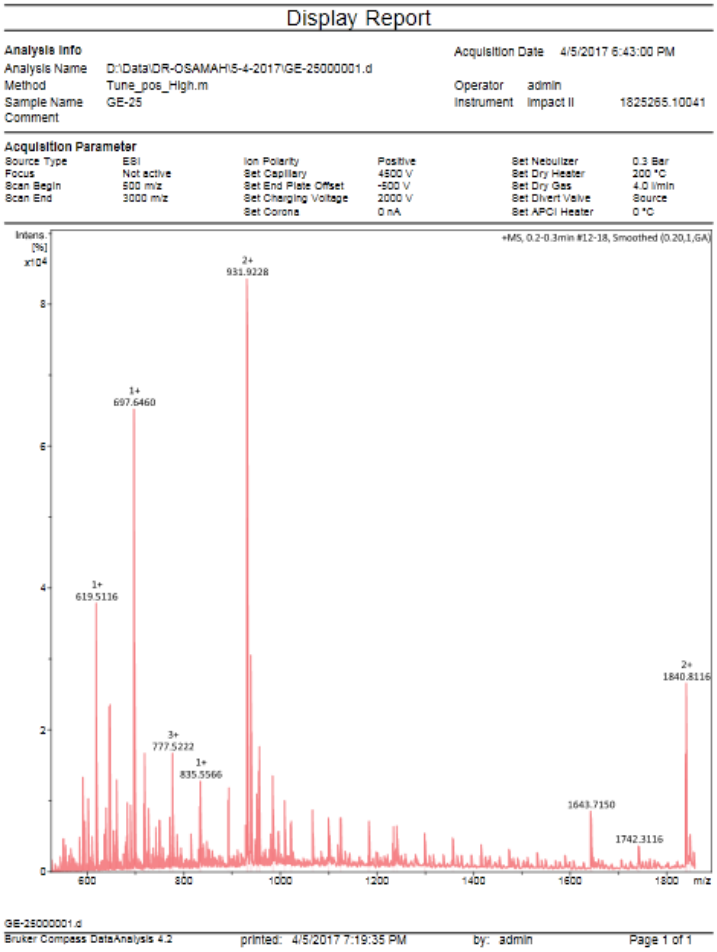


**Figure S28:** HRESI-MS spectrum of compound **3**

 

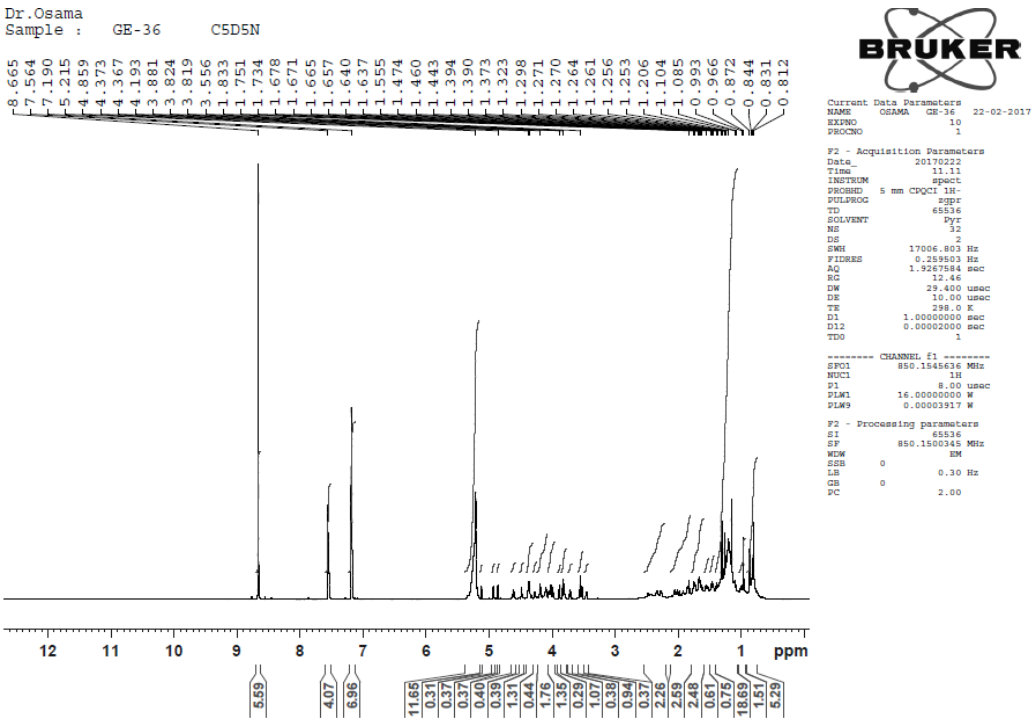


**Figure S29:** $^{1}$H NMR spectrum of compound **4** (Pyridine-$d_5$, 850 MHz)

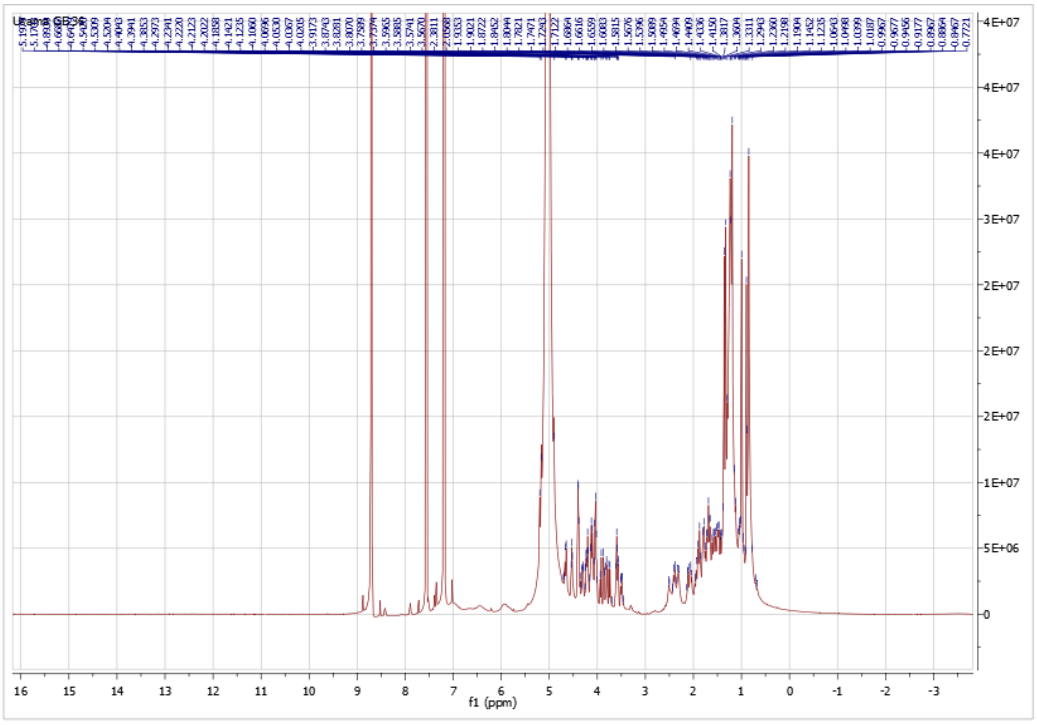


**Figure S30:** $^{1}$H NMR spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

 

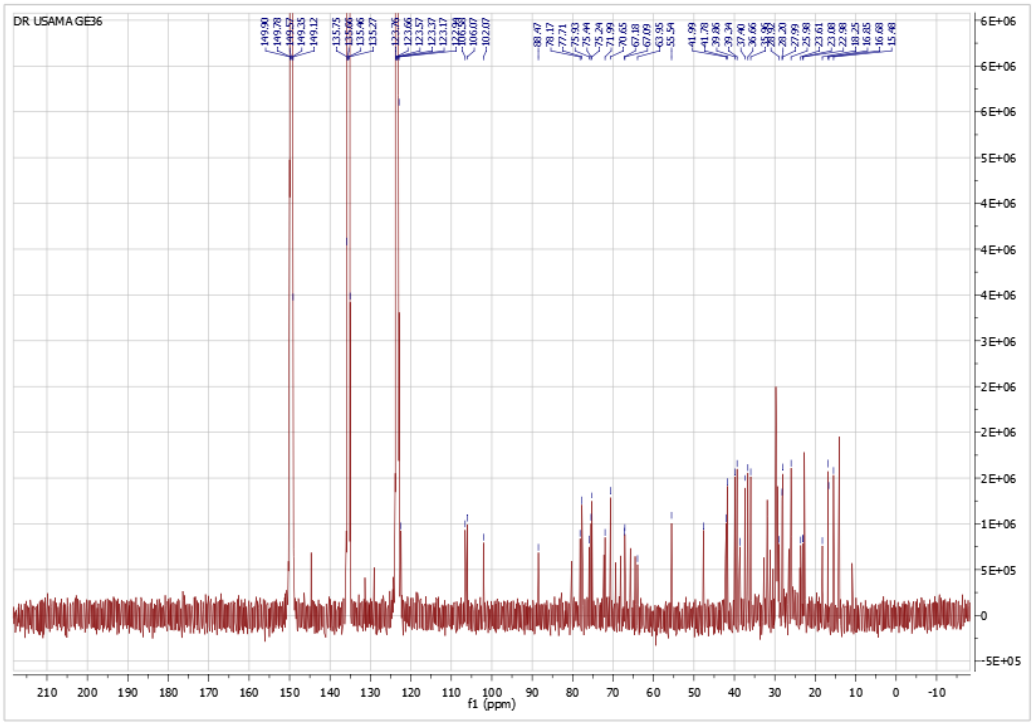


**Figure S31:** $^{13}$C NMR spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

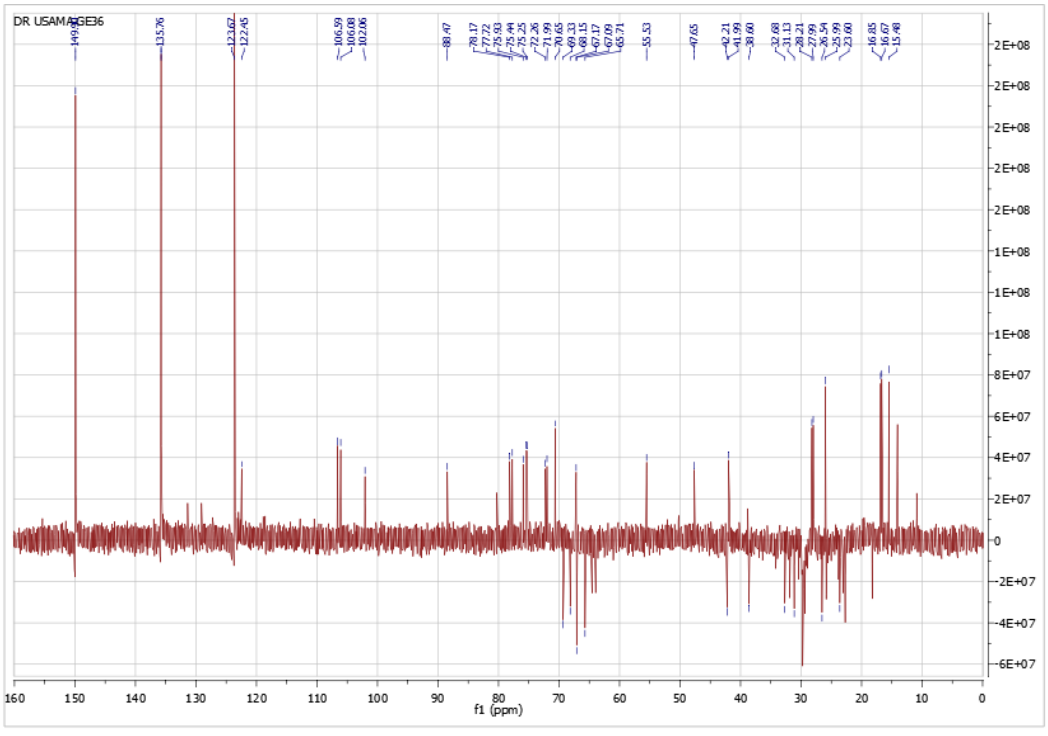


**Figure S32:** DEPT-$^{13}$C NMR spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

 

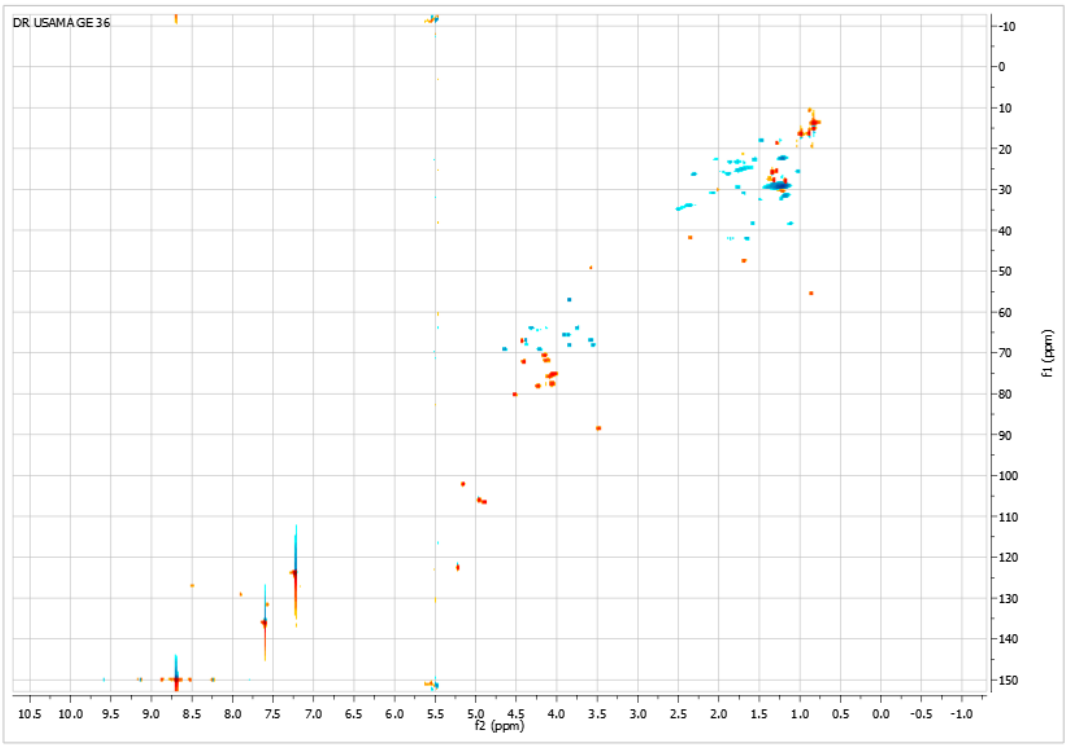


**Figure S33:** HSQC spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

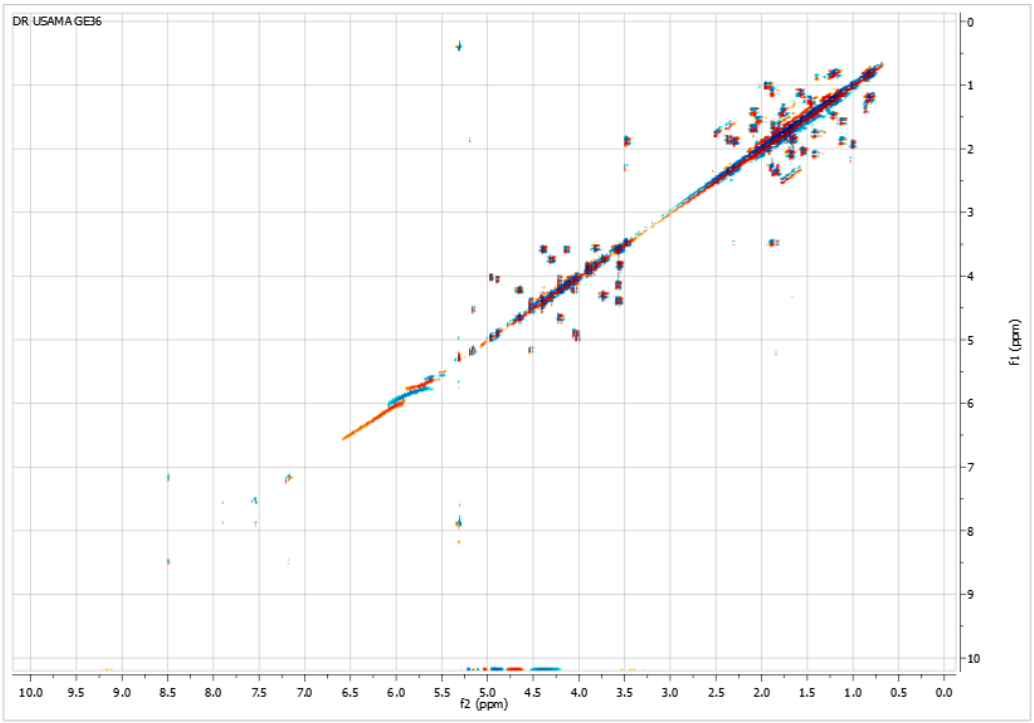


**Figure S34:** COSY spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

 

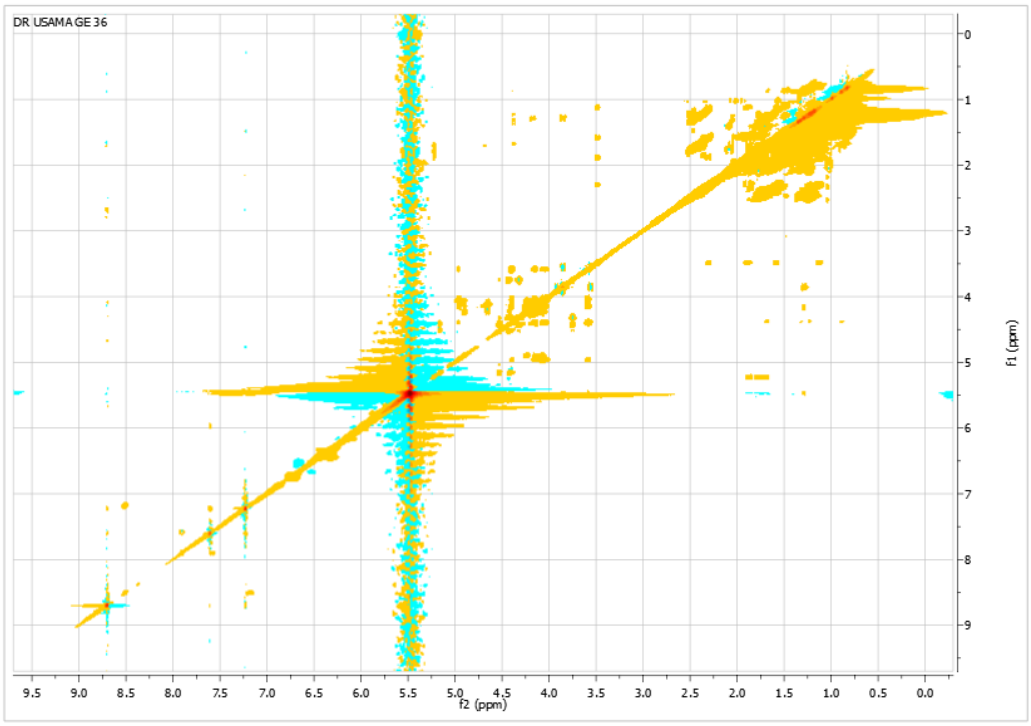


**Figure S35:** TOCSY spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

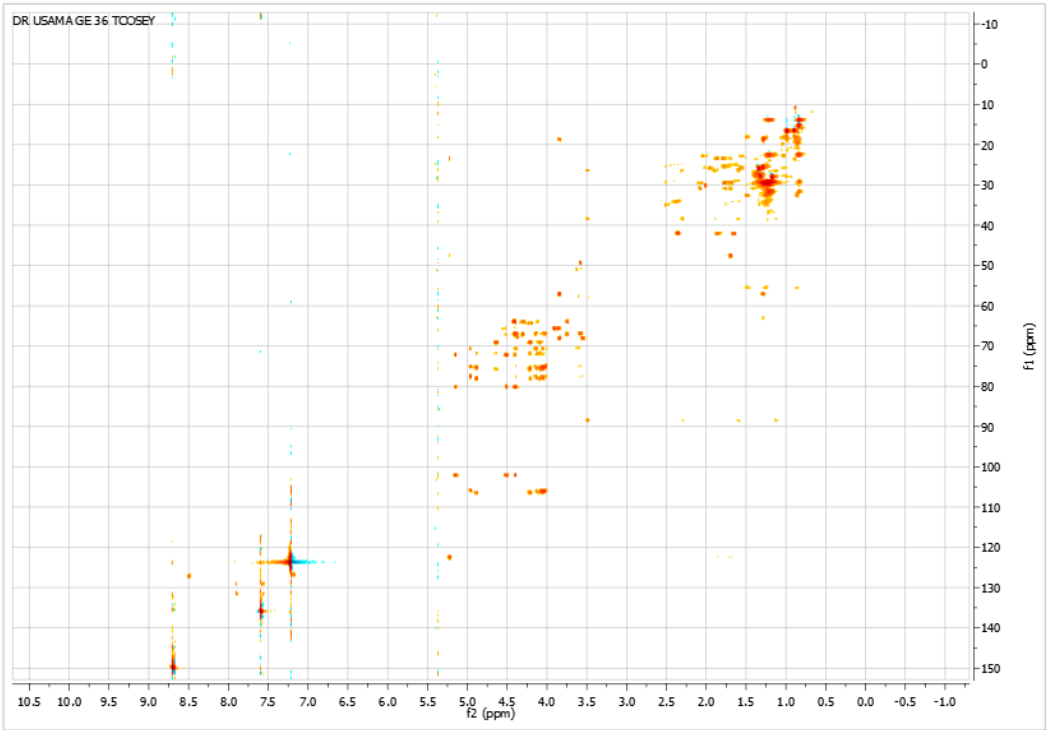


**Figure S36:** HSQC-TOCSY spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

 

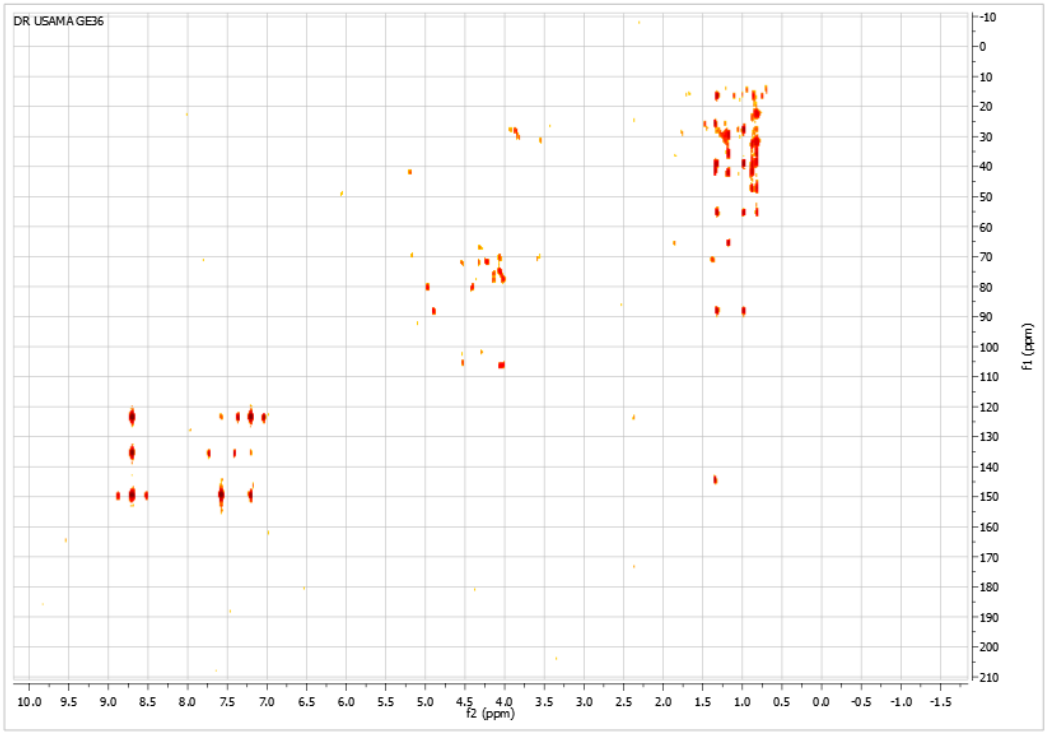


**Figure S37:** HMBC spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

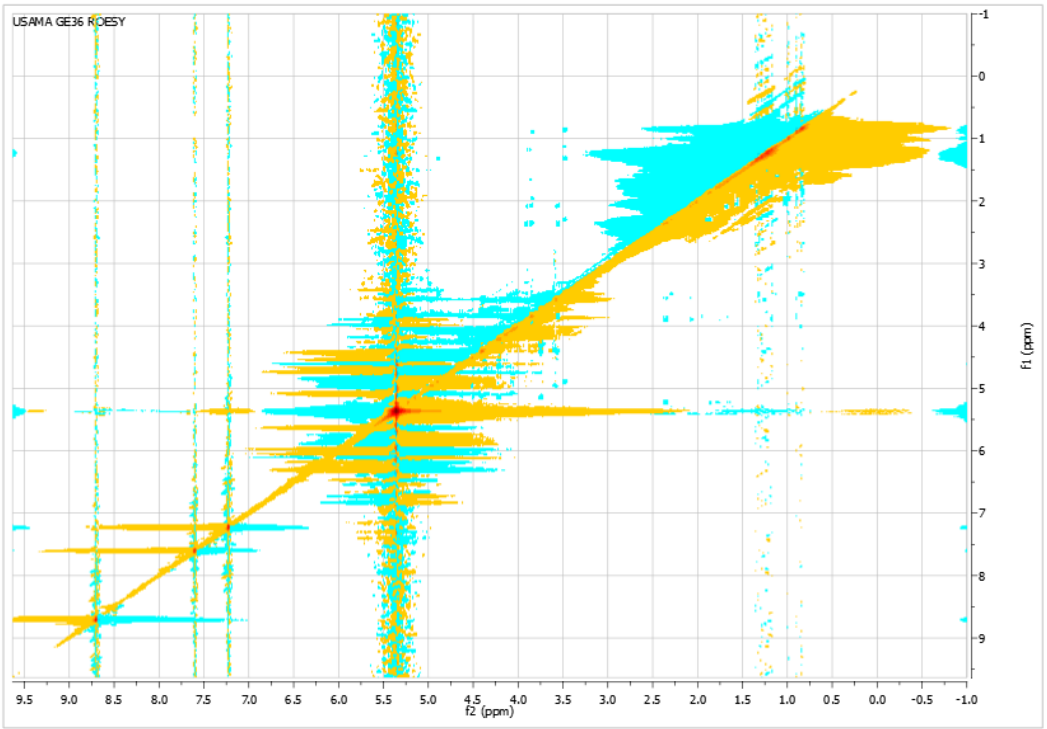


**Figure S38:** ROESY spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

 

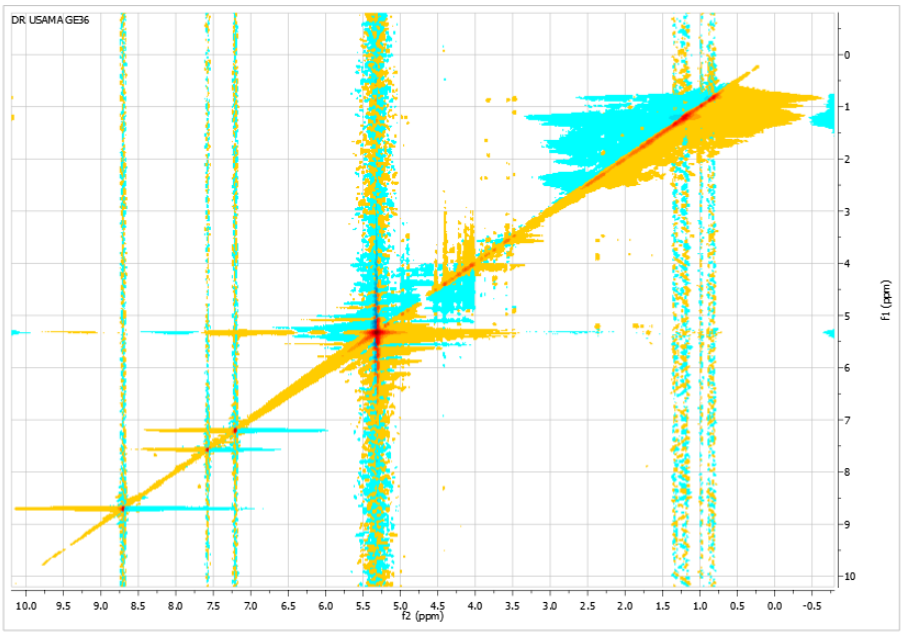


**Figure S39:** NOESY spectrum of compound **4** (Pyridine-$d_5$, 500 MHz)

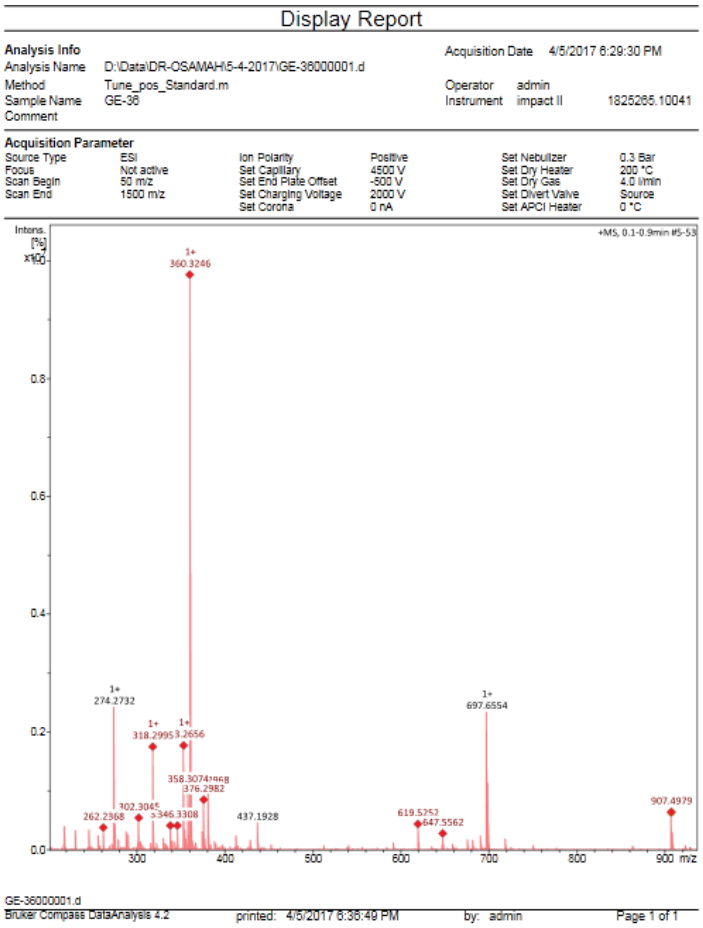


**Figure S40:** HRESI-MS spectrum of compound **4**

 